(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 September 2014 (25.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/149882 A1

(51) International Patent Classification:
C12N 15/11 (2006.01) *C12N 15/79* (2006.01)
C12N 15/63 (2006.01)

(21) International Application Number:
PCT/US2014/021357

(22) International Filing Date:
6 March 2014 (06.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/794,818 15 March 2013 (15.03.2013) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

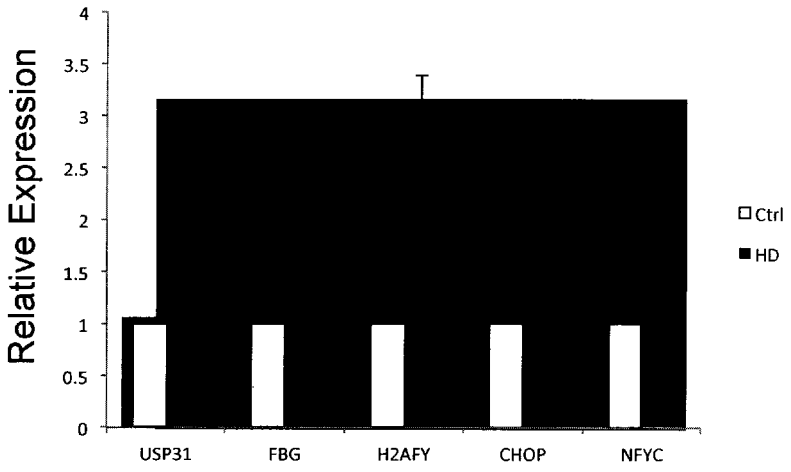
Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) Title: PROMOTER COMPOSITIONS

Fig. 1



(57) Abstract: An isolated promoter sequence comprising a nucleic acid of between 600 and 1700 nucleotides in length having at least 90% identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7.

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PROMOTER COMPOSITIONS

RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application No. 61/794,818 filed March 15, 2013, the entirety of which is incorporated herein by reference.

BACKGROUND

Current approaches to gene therapy for neurodegenerative diseases lack the ability to turn on and shut off expression of the delivered therapeutic gene. In addition, current approaches lack the ability to dose the expression of the therapeutic gene after delivery. There is a current need for regulatable promoters.

SUMMARY

In certain embodiments, the present invention provides an isolated promoter sequence comprising (or consisting of) a nucleic acid of between 500 and 1700 nucleotides in length having at least 90% identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7. In certain embodiments, the promoter has 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7.

In certain embodiments, the present invention provides an expression cassette comprising the promoter described above that is functional in a transformed cell operably linked to a preselected DNA segment encoding a protein or RNA transcript. In certain embodiments, the preselected DNA segment comprises a selectable marker gene or a reporter gene. In certain embodiments, the preselected DNA segment encodes a therapeutic composition. In certain embodiments, the therapeutic composition is an RNAi molecule.

In certain embodiments, the present invention provides a vector comprising the expression cassette described above. In certain embodiments, the vector is an adeno-associated virus (AAV) vector.

In certain embodiments, the present invention provides a transformed cell comprising the expression cassette described above, or the vector described above. In

certain embodiments, the host cell is a eukaryotic cell. In certain embodiments, the eukaryotic cell is an animal cell (e.g. a mammalian cell, such as a human cell).

In certain embodiments, the present invention provides a method for producing transformed cells comprising the steps of (i) introducing into cells a recombinant DNA which comprises a promoter described above operably linked to a DNA segment so as to yield transformed cells, and (ii) identifying or selecting a transformed cell line. In certain embodiments, the recombinant DNA is expressed so as to impart a phenotypic characteristic to the transformed cells. In certain embodiments, the transformed cells exhibit significantly increased expression of a reporter gene when introduced into the cells derived from Huntington's disease patients as compared to cells derived from control individuals.

In certain embodiments, the present invention provides transformed cell made by the method described above.

In certain embodiments, the present invention provides a transformed cell comprising the isolated promoter described above.

In certain embodiments, the present invention provides transformed cell comprising the expression cassette described above.

In certain embodiments, the present invention provides method of treating a neurodegenerative disease in a mammal comprising administering (a) the vector described above, or (b) the transformed cell described above.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Expression analysis for five of the promoters demonstrates upregulation of their endogenous transcripts in Huntington's disease postmortem brain patient material when compared to healthy control individuals.

Figures 2A-2C. Upregulation of the endogenous transcripts in the brain of Huntington's disease mice at 6 weeks (Fig. 2A), 11 weeks (Fig. 2B) and 19 weeks ((Fig. 2C).

Figure 3. Activity of the cloned, gene promoter sequences.

Figure 4. Induction of the cloned, gene promoter sequences as demonstrated by the expression of a reporter gene (Luciferase).

DETAILED DESCRIPTION

The present invention provides the use of gene promoter sequences that can activate, enhance or repress the expression of a genetic sequence at the onset of or during the pathological progression of a neurodegenerative disorder. This invention is directly applicable to the field of gene therapy. It has potential commercial value as a tool in the development of regulated gene therapy approaches to neurodegenerative diseases. This invention provides a means on which to dose or regulate the expression of therapeutic genes during the course of disease based on the state or rate of progression of the neurodegenerative disease. For example, during disease progression, the gene promoter sequences can activate the expression of a therapeutic gene. If the therapy halts the progression of disease, activity of the gene promoter sequence would wane, resulting in reduced/limited therapeutic gene expression. This disease-regulated dynamic approach to therapeutic gene expression is unique and needed in the gene therapy field.

The invention is applicable to the field of gene therapy. It satisfies a need for the regulation and/or dosing of therapeutic genes after delivery into the brain. Gene expression analyses have shown that at the onset and/or during the course of neurodegenerative disease progression, a number of gene promoter sequences act to "turn-on," enhance, repress or "shut-off" the expression of genes. The present inventors have identified and cloned gene promoter sequences that activate and/or enhance gene expression during neurodegenerative disease progression in cell and animal models of neurodegenerative disease. These gene promoter sequences can also activate and/or enhance the expression of a reporter gene during neurodegenerative disease progression in models of neurodegenerative disease. This approach is unique and different from the current systems used to artificially regulate therapeutic gene expression in that it relies on neurodegenerative disease related molecular events to modulate the expression of the therapeutic genes. These events, and thus the activity of the specific gene promoter sequences, are controlled/induced by intracellular and/or extracellular signals that are dependent on the onset and/or progression of disease.

The present inventors have identified, cloned and tested the activity of seven different gene promoter sequences in tissue cultured cells obtained from control individuals or individuals who carry the Huntington's disease (i.e., neurodegenerative disease) gene mutation. The experiments demonstrate that these gene promoter sequences can drive significantly increased expression of a reporter gene when introduced into the cells derived from Huntington's disease patients as compared to cells derived from control individuals. Moreover, introduction of an artificial stressor such as a

proteasome inhibitor or a pro-inflammatory stimulus results in increased activity from the gene promoter sequences as measured by the expression of endogenously controlled transcripts or a reporter gene in cultured cells. In addition, we have detected in the brains of mouse models of Huntington's' disease (i.e., neurodegenerative disease) a disease progression-dependent increase in the activity of these gene promoter sequences as measured by quantitative PCR analysis of the endogenous regulated gene transcripts.

Promoter Sequences

In certain embodiments, the present provides the following promoter sequences that activate and/or enhance gene expression: *ubiquitin specific peptidase 31* (*USP31,* SEQ ID NO:1), *gamma-FBG* (SEQ ID NO:2), *H2A histone family, member Y* (*H2AFY,* SEQ ID NO:3), *Nuclear transcription factor Y, gamma* (*NYFC,* SEQ ID NO:4), *DNA-damage-inducible transcript 3* (*CHOP,* SEQ ID NO:5), *Non-coding 38A RNA* (*38A locus,* SEQ ID NO:6), and *Non-coding 17A RNA* (*17A,* SEQ ID NO:7).

GeneID: *USP31* (SEQ ID NO:1)

GGAGGTCATATACTAGTTGATTTTGGACtaaaggcatcccaaagatgtactatttttttgctcc
ctgcactatttttttttttaatggaaccaacgtttaaaaaataggatttctgccttctcttaaaacatcagatgatctggtaacgctgagctc
ctgttctcacatgggaacaactggctggagctgagtggcagccacctctttagaaagatgttaatttgaagtctgctatagtccccat
cattccttatatttccccccaacactgagacccaagtcacttgaaagtcaatatgatgattcaaacaccaggattcttcactcatttgtg
ggtttgtgacctgtgatatttgtggattcgtgacctttggtttagaggtcaagtatagggggttgtaaataaaacttgggtttgaatcaga
taagaactgtaccaatctcaaagacggtgaattaaagattaaagaaaatattgcatgcttggcataatgtgtggaacacaacagcta
ttgtaaacacccagtaggaatgtaagctgcaagagggcaggtagaaaccgtgctaatttaatccgctggggatatgacatttgatt
ctaatttaataggtttaattctttgcaatatccccagtaactgctgaatgaatgagtgagtgaatgaatgaatggaatgatgaatgatg
ggagagcccggaggagttttaaaggctggaatgaaaaggaaagtagttatgataacagcgtttcgctgggactccaaggtgcag
ccaggaagaaaagcccgctgggacgtgccagggtctgtgactgcgctccgctggcacccagagggtaggtatctgcaacgtc
ccttgggagccagacagggctgacttgcccaggacctgtcagggccatgtccaccagctcttgcacggtctggtaactgacagc
atcctgattcctgcacgttcccgcctctgccatctgaccagcaaagacagagacccacccccggggatccaccatccaacggga
gcccagccttttcctccaagccgggacggcgcccacactagacatggcctccctcgggcttcacgcagcaggaccgcgcccg
cagcctccaggcatcgcgtccggctggagaggcagctaccgtcccacagtgaacatggcggccgaggcggcttcagcgccg
ggcgggcggcgccgggaggcacgtcacgtgagcggctcagctgggcggaccgcgcgcgcgcccgctggccccgccggc
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caaggtaacggcgcctgggtccgggccgccggcggcggcgagcgggaaggagaagcgctccttcagcaagcggctgtttc

ggagcggccgcgctggcggcggcggcgcgggggggccccggggcgtccgggccggccgcgccttcctcgccctcctcgcc
ctcctctgcacgctcggtgggcAGCTTCATGAGCCGCGTTCTCAAG

Gene ID: *gamma-FBG* (SEQ ID NO:2)

TGAGAAGTGAGAGCCTATGAACATGGttgacacagagggacaggaatgtatttccagggtc
attcattcctgggaatagtgaactgggacatgggggaagtcagtctcctcctgccacagccacagattaaaaataataatgttaact
gatccctaggctaaaataatagtgttaactgatccctaagctaagaaagttcttttggtaattcaggtgatggcagcaggacccatct
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aaaaaggaggagcttcaacctgtgtgcaaaatctgggaacctgacagtataggttgggggccaggatgaggaaaaaggaacg
ggaaagacctgcccacccttctggtaaggaggccccgtgatcagctccagccatttgcagtcctggctatcccaggagcttacat
aaagggacaattggagcctgagaggtGACAGTGCTGACACTACAAGGCTCGG

Gene ID: *H2AFY* (SEQ ID NO:3)

CCAAGATCAGCTCTTGGAGGAGTGtagactttaattccacctggtggtccctacagaacggaga
gtccttgcccaaaggcacagagaaggatggaacaacaatgtggtgtgtgggggagggtctctgcacctttcctgacatcttttctt
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ccaggggaaccgggtgcgcagtaattgatcttggggcagaccagggcttggcggtggcctgtatctaaagacagcggggtctc
tgaggcggggcaggggggagttggcattgactggggagggaagagcgatcGCTGGTAACAGCCATTGTG
CCTTC

Gene ID: *NYFC* (SEQ ID NO:4)

GTGCCTGATGTGAGATTTATTGAAACTGGatttgaaaacaattttcctgaattcaagtcgag
ggtttactccactattctcaattactttatcaccagggttataaacccaaaggaagtgagtaacaacagtgtgattttattgaggaccc
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ttacctaatgcactaactctcactgtaggtaattccattcaagacacatttgatgactatacctacaggttttgaagttattcggatggg
gttttgacacttactagctgtggggacttagatcaagttacttaacctttttgagcttcagttttatcatttgtaaaatggatacaatcctg
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cattgcccgactccgtaggagcgcgggggcggctcctgctcttcctggactcctgagcagaggtgtgtgagtgtgcgggagttt
ctgtgcgagggtgatagggaagcggcggcgggggggaggggcagcgcttccCGCCTTCGCCAGAGACCTC
ACTTC

Gene ID: *CHOP* (SEQ ID NO:5)

CTTTTGGGAGATtTACGGGGCTAGAACAGGAGACCACCCcgtttttttgtttgtttg
ttttgttttttgtttttggtgaaacgtagtctcgctctgtcacccaggctggagtgcagtggcgcgatctcggtcactgcaacatccgc
ctccagggttcaagcgattcttctgcctctgcctccagagtagctgggattacaggcgcgcatcaccacacccggctaatttttgta
tttttagtagagacggggtttcaccatgttggtcaggctgatctcgaactcctgacctcaagtgatccgctctcctcagcctcccaaa
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ggataccaactttaaaaagaaaagttcaaaaggcctatgtgcccattagctgggaggggccaagaaatatgggagtcccttatag
tgggggtaaaacggcgggtaaagctaggtgggcggaacagcagcttctgggggagacaagcggcaaagaggctcacgacc
gactaggggcgaccaaggctgatagccgttggggccgttgggcgccgggagctggcgccccgccctctctcctctccccac
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ggatgcgagggacggggcggggccaatgccggcgtgccactttctgattggtaggttttggggtcccgcccctgagaggagg
gcaaggccatggtaaaagattacagccaggcgctcCCGAGGTCAGAGACTTAAGTCTAAGgcaCTG
AGCGTATC

Gene ID: *38A locus* (SEQ ID NO:6)

TAATAACAACATATCTGAAAAAGACGCtttaaaatcccatttatgaaagcataaaaatagtta
gaaataaatttaaccataaaggtgaaatatttgtataccgataactataaacctttgataaaaaaagttgaagaagAcacatataaat
agaataatattctgtgttcatgaatcaaaaaatttaacaatgttaaaatgtctgtattaaccaaagcaatatacaaattcaatgcaatttc
tatcaaaatttcaaggatatgcatcacagaaatagaaaaaaaattcttgaaattcatatggaaccacagacacataaaaacagaata
ggcaaaggaacaatgagaaagcaaaacaaagcttgaggcatcacacttcctaagttaaaattatattgcaaagctacagtaatca
aaaacagtatacaaatggcatgaaaacgaaaatgtggaccaacggaacagaatatagagagccagaaacttaactaaTTTTc
aacaagGGTACCAACAGGACACCCTGAA

Gene ID: *17A locus* (SEQ ID NO:7)

ATTTCCACCAGCAACACACAAGGGTTCCAGTTTCTCCACATCTTCACCA
ACACTTGTGGTTTTGCTTTTTGGTAATAGCCATGCTAATGGGTGTGAACAAGA
AGTGCTTTAAGCATCTCCTAAAGCGGAAGAAACTGAGGCCCAGAGAAGGGAA
GAATCACACGAGAGATTGAGGTCACAAGCAAGTCAGTGATAGAGCAGGACCT
GGAAGCTGGATCCCCTAACCCCAGCCTAGTTCTTGCTACTAAAACCCAAAATC
CAGTTTCCATTGCTATATGTCAGAGGGTGCACAGCCATGGCCACAGGCCAGA
TACAGACTTTAAGTTTATTTGGTTTGATCCTTACTTTCTTTTTTTTTTAATTCAA
AATAGTATCAACATTTAAAAATTAGGGGATTTTATATTTTAAAAGTCTAAATT
TCTGATTTCTCCCCTCAAAAATCAGAAGGTCTGGTAACCCTTGACCCACATTC
TAACTCAGCAACCAACTATTACTGTCTTTTGTTTTGTTTTGTTCTGTTGAGACA
AGGTCTTGTTCTGTCACCCAGGCTGGAGTACGGTGGCGTGATCACGGCTCACT
GCAGTCTTGAACTCCTGGGCTCAAGCAAGCCCCCCGTCTTGGCCTCCCAAAGC
TCTGGGATTACAGGTGTGAGCCCACGCCCAGCCCTATCATTCTGTAATATCCT
TCCACACAGGCTAGTTCACACACTGGCTGGTCCTGGTAACACTGGAGTTTGCA
GCCCTTTGCTTTTCACATCCATAGATATTCCTCATTCTGAGTGTCAGTAGACAC
ATAGTTACGTGTAACATCATAGGCAGGTTCCATACTTCTTTCCTCTTTCCTTTA
CTCTATATTGTCTTTGAATATCTTAGCTATTTCCTCACCATAAAAGTGAAATAA
TGTTGCAAATAAATAGTGCAAAATATTAACAAAGACACAATTGAATAGCCTG

"Promoter" refers to a nucleotide sequence, usually upstream (5') to its coding sequence, that controls the expression of the coding sequence by providing the recognition for RNA polymerase and other factors required for proper transcription. "Promoter" includes a minimal promoter that is a short DNA sequence comprised of a TATA-box and other sequences that serve to specify the site of transcription initiation, to

which regulatory elements are added for control of expression. "Promoter" also refers to a nucleotide sequence that includes a minimal promoter plus regulatory elements that is capable of controlling the expression of a coding sequence or functional RNA. This type of promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a DNA sequence that can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue specificity of a promoter. It is capable of operating in both orientations (normal or flipped), and is capable of functioning even when moved either upstream or downstream from the promoter. Both enhancers and other upstream promoter elements bind sequence-specific DNA-binding proteins that mediate their effects. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even be comprised of synthetic DNA segments. A promoter may also contain DNA sequences that are involved in the binding of protein factors that control the effectiveness of transcription initiation in response to physiological or developmental conditions.

As used herein, "biologically active" means that the promoter has at least about 0.1%, 10%, 25%, 50%, 75%, 80%, 85%, even 90% or more, e.g. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the activity of the CCT promoter comprising SEQ ID NO:1 or SEQ ID NO:2. The activity of a promoter can be determined by methods well known to the art. For example, see Sambrook et al., Molecular Cloning: A Laboratory Manual (1989). Promoters of the present invention that are not identical to SEQ ID NO:1 or SEQ ID NO:2, but retain comparable biological activity, are called variant promoters. The nucleotide sequences of the invention include both naturally occurring sequences as well as recombinant forms.

The invention encompasses isolated or substantially purified nucleic acid compositions. In the context of the present invention, an “isolated” or “purified” DNA molecule or RNA molecule is a DNA molecule or RNA molecule that exists apart from its native environment and is therefore not a product of nature. An isolated DNA molecule or RNA molecule may exist in a purified form or may exist in a non-native environment such as, for example, a transgenic host cell. For example, an “isolated” or “purified” nucleic acid molecule or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when

chemically synthesized. In one embodiment, an "isolated" nucleic acid is free of sequences that naturally flank the nucleic acid (i.e., sequences located at the 5′ and 3′ ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Fragments and variants of the disclosed nucleotide sequences are also encompassed by the present invention. By "fragment" or "portion" is meant a full length or less than full length of the nucleotide sequence.

The term "nucleic acid" refers to deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and polymers thereof in either single- or double-stranded form, composed of monomers (nucleotides) containing a sugar, phosphate and a base that is either a purine or pyrimidine. Unless specifically limited, the term encompasses nucleic acids containing known analogs of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues. A "nucleic acid fragment" is a portion of a given nucleic acid molecule.

"Naturally occurring," "native," or "wild-type" is used to describe an object that can be found in nature as distinct from being artificially produced. For example, a protein or nucleotide sequence present in an organism (including a virus), which can be isolated from a source in nature and that has not been intentionally modified by a person in the laboratory, is naturally occurring.

Vectors and Expression Cassettes

In certain embodiments, the present invention provides vectors and expression cassettes containing the promoters described above.

Vectors

A "vector" is defined to include, *inter alia*, any viral vector, as well as any plasmid, cosmid, phage or binary vector in double or single stranded linear or circular

form that may or may not be self-transmissible or mobilizable, and that can transform prokaryotic or eukaryotic host either by integration into the cellular genome or exist extrachromosomally (*e.g.*, autonomous replicating plasmid with an origin of replication).

The selection and optimization of a particular expression vector for expressing a specific therapeutic composition (e.g., a protein) in a cell can be accomplished by obtaining the nucleic acid sequence encoding the protein, possibly with one or more appropriate control regions (*e.g.*, promoter, insertion sequence); preparing a vector construct comprising the vector into which is inserted the nucleic acid sequence encoding the protein; transfecting or transducing cultured cells *in vitro* with the vector construct; and determining whether the protein is present in the cultured cells.

Vectors for cell gene therapy include viruses, such as replication-deficient viruses. Replication-deficient retroviruses are capable of directing synthesis of all virion proteins, but are incapable of making infectious particles. Accordingly, these genetically altered retroviral expression vectors have general utility for high-efficiency transduction of nucleic acid sequences in cultured cells, and specific utility for use in the method of the present invention. Such retroviruses further have utility for the efficient transduction of nucleic acid sequences into cells *in vivo*. Retroviruses have been used extensively for transferring nucleic acid material into cells. Protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous nucleic acid material into a plasmid, transfection of a packaging cell line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with the viral particles) are well known in the art.

An advantage of using retroviruses for gene therapy is that the viruses insert the nucleic acid sequence encoding the target protein into the host cell genome, thereby permitting the nucleic acid sequence encoding the target protein to be passed on to the progeny of the cell when it divides. Promoter sequences in the LTR region have can enhance expression of an inserted coding sequence in a variety of cell types.

Another viral candidate useful as an expression vector for transformation of cells is an adenovirus (Ad), which is a double-stranded DNA virus. The adenovirus is infective in a wide range of cell types, including, for example, muscle and endothelial cells. Adenoviruses are double-stranded linear DNA viruses with a 36 kb genome. Several features of adenovirus have made them useful as transgene delivery vehicles for therapeutic applications, such as facilitating *in vivo* gene delivery. Recombinant adenovirus vectors have been shown to be capable of efficient *in situ* gene transfer to

parenchymal cells of various organs, including the lung, brain, pancreas, gallbladder, and liver. This has allowed the use of these vectors in methods for treating inherited genetic diseases, such as cystic fibrosis, where vectors may be delivered to a target organ.

Like the retrovirus, the adenovirus genome is adaptable for use as an expression vector for gene therapy, *i.e.*, by removing the genetic information that controls production of the virus itself. Because the adenovirus functions in an extrachromosomal fashion, the recombinant adenovirus does not have the theoretical problem of insertional mutagenesis.

Several approaches traditionally have been used to generate the recombinant adenoviruses. One approach involves direct ligation of restriction endonuclease fragments containing a nucleic acid sequence of interest to portions of the adenoviral genome. Alternatively, the nucleic acid sequence of interest may be inserted into a defective adenovirus by homologous recombination results. The desired recombinants are identified by screening individual plaques generated in a lawn of complementation cells.

Examples of appropriate vectors include DNA viruses (e.g., adenoviruses), lentiviral, adeno-associated viral (AAV), poliovirus, HSV, or murine Moloney-based viral vectors, viral vectors derived from Harvey Sarcoma virus, ROUS Sarcoma virus, MPSV or hybrid transposon based vectors. In one embodiment, the vector is AAV. AAV is a small nonpathogenic virus of the parvoviridae family. AAV is distinct from the other members of this family by its dependence upon a helper virus for replication. The approximately 5 kb genome of AAV consists of one segment of single stranded DNA of either plus or minus polarity. The ends of the genome are short inverted terminal repeats which can fold into hairpin structures and serve as the origin of viral DNA replication. Physically, the parvovirus virion is non-enveloped and its icosohedral capsid is approximately 20 nm in diameter.

To-date many serologically distinct AAVs have been identified and have been isolated from humans or primates. For example, the genome of AAV2 is 4680 nucleotides in length and contains two open reading frames (ORFs). The left ORF encodes the non-structural Rep proteins, Rep 40, Rep 52, Rep 68 and Rep 78, which are involved in regulation of replication and transcription in addition to the production of single-stranded progeny genomes. Rep68/78 has also been shown to possess NTP binding activity as well as DNA and RNA helicase activities. The Rep proteins possess a nuclear localization signal as well as several potential phosphorylation sites. Mutation of one of these kinase sites resulted in a loss of replication activity.

The ends of the genome are short inverted terminal repeats (ITR) which have the potential to fold into T-shaped hairpin structures that serve as the origin of viral DNA replication. Within the ITR region two elements have been described which are central to the function of the ITR, a GAGC repeat motif and the terminal resolution site (trs). The repeat motif has been shown to bind Rep when the ITR is in either a linear or hairpin conformation. This binding serves to position Rep68/78 for cleavage at the trs which occurs in a site- and strand-specific manner. AAV vectors have several features that make it an attractive vector for gene transfer, such as possessing a broad host range, are capable of transduce both dividing and non-dividing cells *in vitro* and *in vivo*, and are capable of maintaining high levels of expression of transduced genes.

In certain embodiments, the viral vector is an AAV vector. An "AAV" vector refers to an adeno-associated virus, and may be used to refer to the naturally occurring wild-type virus itself or derivatives thereof. The term covers all subtypes, serotypes and pseudotypes, and both naturally occurring and recombinant forms, except where required otherwise. As used herein, the term "serotype" refers to an AAV which is identified by and distinguished from other AAVs based on capsid protein reactivity with defined antisera, e.g., there are eight known serotypes of primate AAVs, AAV1 to AAV8. For example, serotype AAV9 is used to refer to an AAV which contains capsid proteins encoded from the cap gene of AAV9 and a genome containing 5′ and 3′ ITR sequences from the same AAV9 serotype. In certain embodiments, the AAV vector is AAV9.

The abbreviation "rAAV" refers to recombinant adeno-associated virus, also referred to as a recombinant AAV vector (or "rAAV vector"). In one embodiment, the AAV expression vectors are constructed using known techniques to at least provide as operatively linked components in the direction of transcription, control elements including a transcriptional initiation region, the DNA of interest and a transcriptional termination region. The control elements are selected to be functional in a mammalian cell. The resulting construct which contains the operatively linked components is flanked (5′ and 3′) with functional AAV ITR sequences.

By "adeno-associated virus inverted terminal repeats" or "AAV ITRs" is meant the art-recognized regions found at each end of the AAV genome which function together in cis as origins of DNA replication and as packaging signals for the virus.

The nucleotide sequences of AAV ITR regions are known. As used herein, an "AAV ITR" need not have the wild-type nucleotide sequence depicted, but may be altered, e.g., by the insertion, deletion or substitution of nucleotides. Additionally, the

AAV ITR may be derived from any of several AAV serotypes, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV7, etc. Furthermore, 5′ and 3′ ITRs which flank a selected nucleotide sequence in an AAV vector need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended, i.e., to allow for excision and rescue of the sequence of interest from a host cell genome or vector.

Nucleic acids encoding therapeutic compositions can be engineered into an AAV vector using standard ligation techniques, such as those described in Sambrook and Russell, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press Cold Spring Harbor, NY (2001). For example, ligations can be accomplished in 20 mM Tris-Cl pH 7.5, 10 mM MgCl2, 10 mM DTT, 33 μg/ml BSA, 10 mM-50 mM NaCl, and either 40 μM ATP, 0.01-0.02 (Weiss) units T4 DNA ligase at 0°C (for "sticky end" ligation) or 1 mM ATP, 0.3-0.6 (Weiss) units T4 DNA ligase at 14°C (for "blunt end" ligation). Intermolecular "sticky end" ligations are usually performed at 30-100 μg/ml total DNA concentrations (5-100 nM total end concentration). AAV vectors which contain ITRs have been described in, e.g., U.S. Pat. No. 5,139,941. In particular, several AAV vectors are described therein which are available from the American Type Culture Collection ("ATCC") under Accession Numbers 53222, 53223, 53224, 53225 and 53226.

In certain embodiments, the adeno-associated virus packages a full-length genome, i.e., one that is approximately the same size as the native genome, and is not too big or too small. In certain embodiments the AAV is not a self-complementary AAV vector.

The viral vector further includes a promoter for controlling transcription of the heterologous gene. The promoter may be an inducible promoter for controlling transcription of the therapeutic composition. The expression system is suitable for administration to the mammalian recipient.

In certain embodiments, viral particles are administered. Viral particles are heat stable, resistant to solvents, detergents, changes in pH, temperature, and can be concentrated on CsCl gradients. AAV is not associated with any pathogenic event, and transduction with AAV vectors has not been found to induce any lasting negative effects on cell growth or differentiation. The ITRs have been shown to be the only cis elements required for packaging allowing for complete gutting of viral genes to create vector systems.

In certain embodiments, the present invention provides a vector containing an expression cassette comprising a promoter operably linked to a target sequence. "Expression cassette" as used herein means a nucleic acid sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, which includes a promoter operably linked to the nucleotide sequence of interest that may be operably linked to termination signals. The coding region usually codes for a functional RNA of interest, for example an RNAi molecule. The expression cassette including the nucleotide sequence of interest may be chimeric.

Certain embodiments of the present invention provide a vector that encodes a target molecule, such as an isolated RNAi molecule. As used herein the term "encoded by" is used in a broad sense, similar to the term "comprising" in patent terminology. RNAi molecules include siRNAs, shRNAs and other small RNAs that can or are capable of modulating the expression of a target gene, for example via RNA interference. Such small RNAs include without limitation, shRNAs and miroRNAs (miRNAs).

"Operably-linked" refers to the association of nucleic acid sequences on single nucleic acid fragment so that the function of one of the sequences is affected by another. For example, a regulatory DNA sequence is said to be "operably linked to" or "associated with" a DNA sequence that codes for an RNA or a polypeptide if the two sequences are situated such that the regulatory DNA sequence affects expression of the coding DNA sequence (i.e., that the coding sequence or functional RNA is under the transcriptional control of the promoter). Coding sequences can be operably-linked to regulatory sequences in sense or antisense orientation. Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. Generally, "operably linked" means that the DNA sequences being linked are contiguous. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. Additionally, multiple copies of the nucleic acid encoding enzymes may be linked together in the expression vector. Such multiple nucleic acids may be separated by linkers.

"Expression" refers to the transcription and/or translation of an endogenous gene or a transgene in cells. For example, in the case of antisense constructs, expression may refer to the transcription of the antisense DNA only. In addition, expression refers to the transcription and stable accumulation of sense (mRNA) or functional RNA. Expression may also refer to the production of protein.

"Expression cassette" as used herein means a DNA sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operably linked to the nucleotide sequence of interest that is operably linked to termination signals. It also typically comprises sequences required for proper translation of the nucleotide sequence. The coding region usually codes for a protein of interest but may also code for a functional RNA of interest, for example antisense RNA or a nontranslated RNA, in the sense or antisense direction. The expression cassette comprising the nucleotide sequence of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette may also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Such expression cassettes will comprise the transcriptional initiation region linked to a nucleotide sequence of interest. Such an expression cassette may be provided with a plurality of restriction sites for insertion of the gene of interest to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

The present disclosure also provides a mammalian cell containing a vector described herein. The cell may be human, and may be from brain, spleen, kidney, lung, heart, or liver. The cell type may be a stem or progenitor cell population.

Nucleic Acids Encoding Therapeutic Composition

The present invention provides a method of administering a therapeutic composition. In certain embodiments, the therapeutic composition is a transgene, which is a gene encoding a polypeptide that is foreign to the retrovirus from which the vector is primarily derived and has a useful biological activity in the organism into which it is administered (e.g., a therapeutic gene). As used herein, the term "therapeutic gene" refers to a gene whose expression is desired in a cell to provide a therapeutic effect, e.g., to treat a disease.

Methods of Use

The present disclosure provides a method of treating a neurodegenerative disease such as a genetic disease, such as Huntington's disease, ALS, hereditary spastic hemiplegia, primary lateral sclerosis, spinal muscular atrophy, Kennedy's disease, Alzheimer's disease, a polyglutamine repeat disease, or focal exposure such as Parkinson's disease by administering a vector containing the isolated promoters described above.

Certain aspects of the disclosure relate to polynucleotides, polypeptides, vectors, and genetically engineered cells (modified *in vivo*), and the use of them. In particular, the disclosure relates to a method for gene or protein therapy that is capable of both systemic delivery of a therapeutically effective dose of the therapeutic agent.

The invention will now be illustrated by the following non-limiting Example.

Example 1

For this experiment, post-mortem human brain tissue obtained from 6 different Huntington's disease patients or 5 control healthy individuals were used to analyze the activity of the 5 different polymerase 2 promoters. RNA was extracted and subjected to quantitative PCR in order to measure the amount of endogenous RNA transcript produced by these promoters. We found significant upregulation for all five promoters in the brains of Huntington's disease patients when compared to the healthy individuals. **Fig. 1**.

Wild type and HD mice were sacrificed at 6 and 11 weeks (pre-symptomatic) and at 19 weeks (symptomatic) old. Total RNA was isolated from striatum samples and expression of mouse Nfyc, H2afy, Usp31 and Chop genes were determined by using real time Q-PCR. At 6 and 11 weeks of age none of the genes were up-regulated, whereas at 19 week of age the expression of Nfyc and H2afy was significantly upregulated. Number of mice tested are 4 per group. **Figs. 2A-2C.**

Each of the gene promoter sequences were cloned upstream of a reporter gene encoding for Firefly Luciferase. These constructs were transiently expressed in human cultured cells in order to validate their transcriptional activity at basal conditions. **Fig. 3.**

Promoter constructs shown in Attachment 4 were transiently transfected into control or Huntington's disease-derived cultured fibroblast cells. We observed increased activity of the cloned gene promoter sequences in Huntington's disease fibroblasts when compared to control fibroblast cells. **Fig. 4.**

All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (*i.e.*, meaning "including, but not limited to") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

Embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

WHAT IS CLAIMED IS:

1. An isolated promoter sequence comprising a nucleic acid of between 500 and 1700 nucleotides in length having at least 90% identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7.

2. An isolated promoter sequence consisting of a nucleic acid of between 500 and 1700 nucleotides in length having at least 90% identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7.

3. The isolated promoter sequence of claim 1 or 2, wherein the nucleic acid has at least 95% identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7.

4. An isolated promoter sequence comprising a nucleic acid of between 1500 and 1700 nucleotides in length having at least 90% identity to SEQ ID NO:1.

5. An isolated promoter sequence comprising a nucleic acid of between 600 and 800 nucleotides in length having at least 90% identity to SEQ ID NO:2.

6. An isolated promoter sequence comprising a nucleic acid of between 1300 and 1400 nucleotides in length having at least 90% identity to SEQ ID NO:3.

7. An isolated promoter sequence comprising a nucleic acid of between 1300 and 1500 nucleotides in length having at least 90% identity to SEQ ID NO:4.

8. An isolated promoter sequence comprising a nucleic acid of between 1000 and 1200 nucleotides in length having at least 90% identity SEQ ID NO:5.

9. An isolated promoter sequence comprising a nucleic acid of between 500 and 700 nucleotides in length having at least 90% identity to SEQ ID NO:6.

10. An isolated promoter sequence comprising a nucleic acid of between 900 and 1700 nucleotides in length having at least 90% identity to SEQ ID NO:7.

11. An expression cassette comprising the promoter of any one of claims 1 to 10 that is functional in a transformed cell operably linked to a preselected DNA segment encoding a protein or RNA transcript.

12. The expression cassette of claim 11, wherein the preselected DNA segment comprises a selectable marker gene or a reporter gene.

13. The expression cassette of claim 11, wherein the preselected DNA segment encodes a therapeutic composition.

14. The expression cassette of claim 13, wherein therapeutic composition is an RNAi molecule.

15. A vector comprising the expression cassette of any one of claims 11 to 14.

16. The vector of claim 15, wherein the vector is an adeno-associated virus (AAV) vector.

17. A transformed cell comprising the expression cassette of any one of claims 11 to 14, or the vector of claim 15 or 16.

18. The transformed cell of claim 17, wherein the host cell is a eukaryotic cell.

19. The transformed cell of claim 18, wherein the eukaryotic cell is an animal cell.

20. The transformed cell of claim 19, wherein the animal cell is a mammalian cell.

21. The transformed cell of claim 20, wherein the mammalian cell is a human cell.

22. A method for producing transformed cells comprising the steps of (i) introducing into cells a recombinant DNA which comprises a promoter of any one of claims 1 to 10

operably linked to a DNA segment so as to yield transformed cells, and (ii) identifying or selecting a transformed cell line.

23. The method of claim 22, wherein the recombinant DNA is expressed so as to impart a phenotypic characteristic to the transformed cells.

24. The method of claim 22, wherein the transformed cells exhibit significantly increased expression of a reporter gene when introduced into the cells derived from Huntington's disease patients as compared to cells derived from control individuals.

25. A transformed cell made by the method of any one of claims 22 to 24.

26. A transformed cell comprising the isolated promoter of any one of claims 1 to 10.

27. A transformed cell comprising the expression cassette of any one of claims 11 to 14.

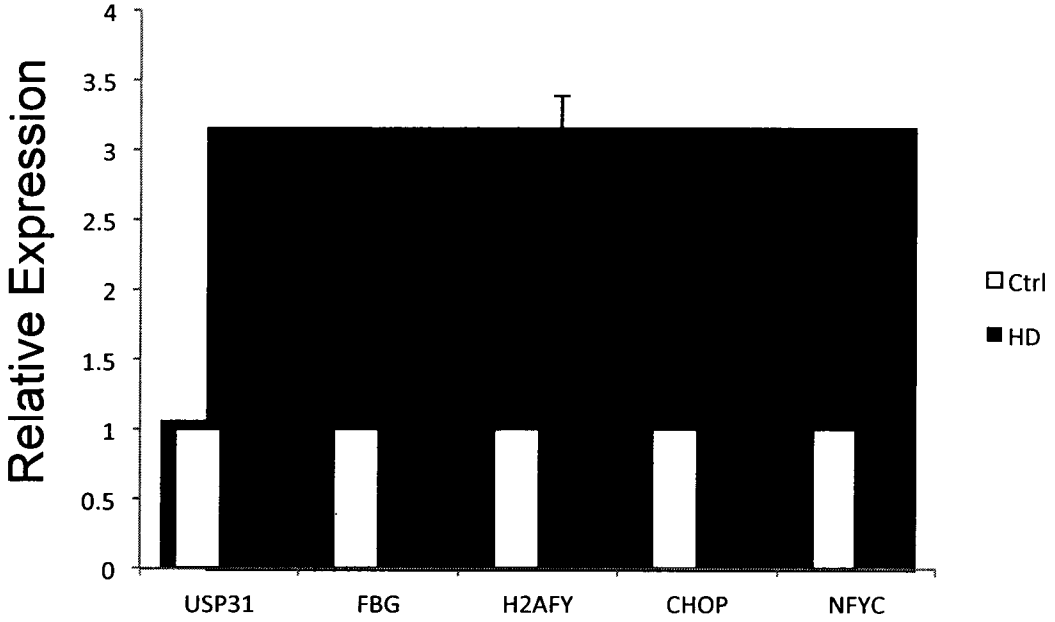
28. A method of treating a neurodegenerative disease in a mammal comprising administering

(a) the vector of claim 15 or 16, or

(b) the transformed cell of any one of claims 25 to 27.

29. A use of the isolated promoter of any one of claims 1 to 10, wherein the promoter activates, enhance or repress the expression of a genetic sequence at onset of or during pathological progression of a neurodegenerative disorder.

Fig. 1


Relative Expression
4
3.5
3
2.5
2
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1
0.5
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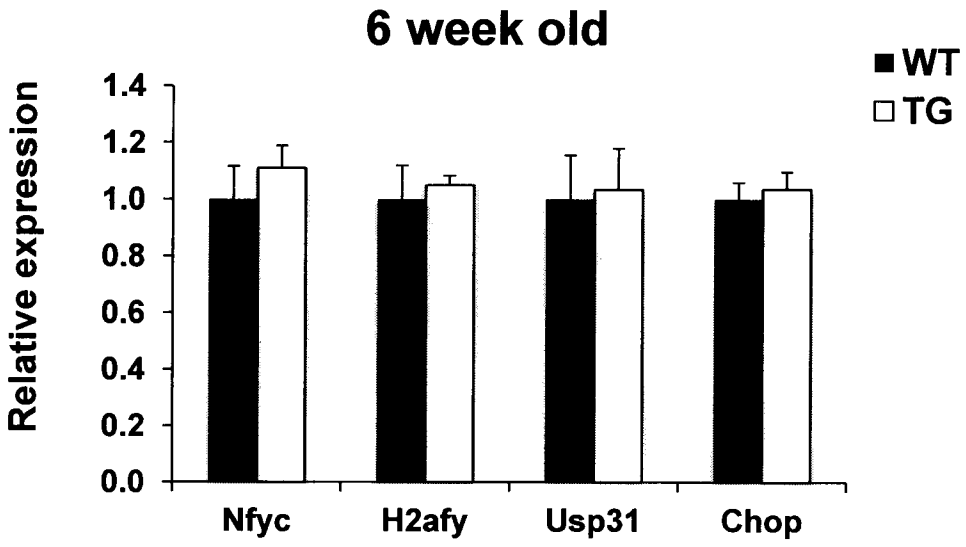
USP31
FBG
H2AFY
CHOP
NFYC
Ctrl
HD

Fig. 2A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Relative expression
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1.2
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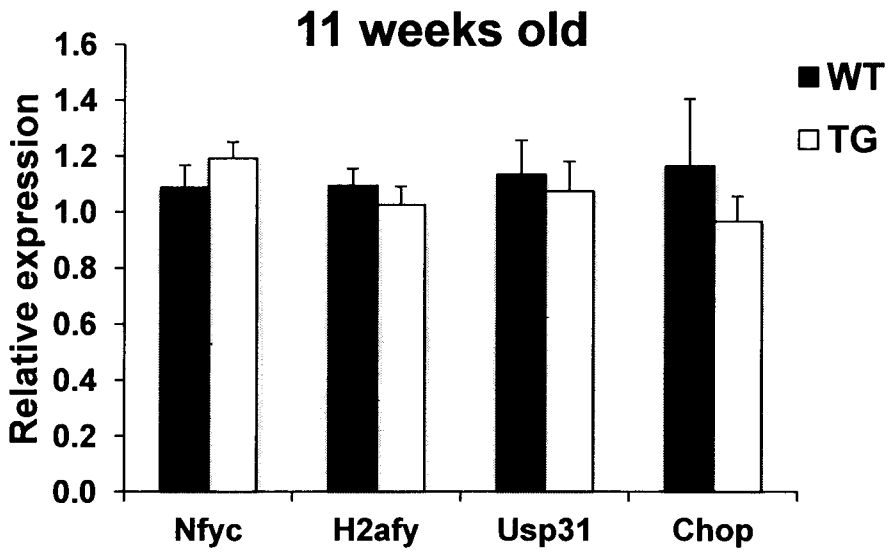
H2afy
Usp31
Chop
WT
TG

Fig. 2B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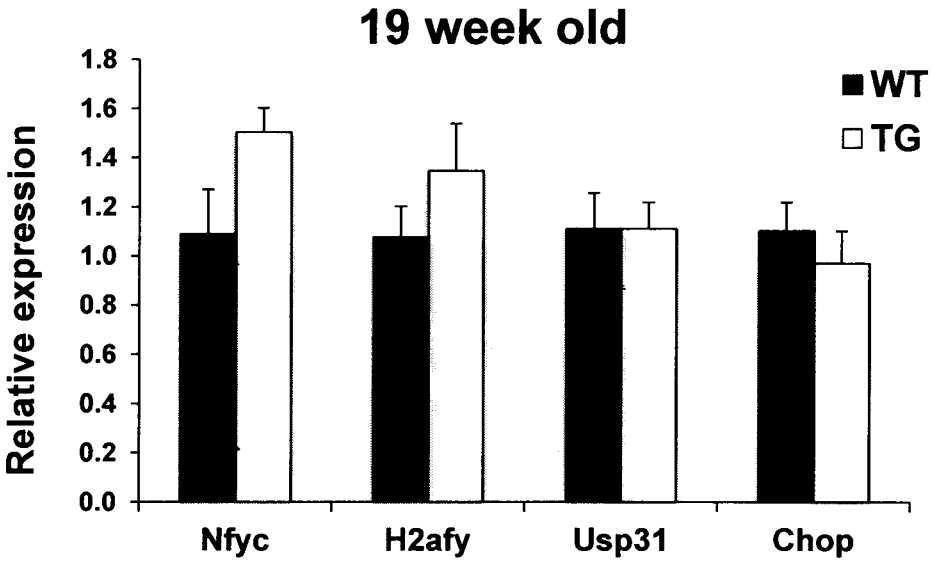
Relative expression
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WT
TG
Nfyc
H2afy
Usp31
Chop

Fig. 2C


19 week old
Relative expression
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1.6
1.4
1.2
1.0
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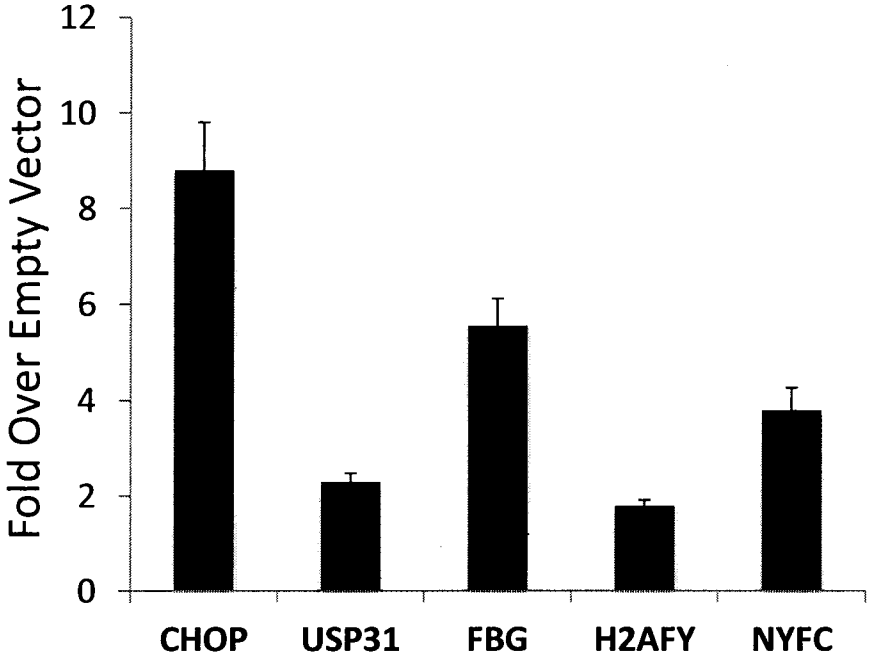
0.4
0.2
0.0
WT
TG
Nfyc
H2afy
Usp31
Chop

Fig. 3


Fold Over Empty Vector
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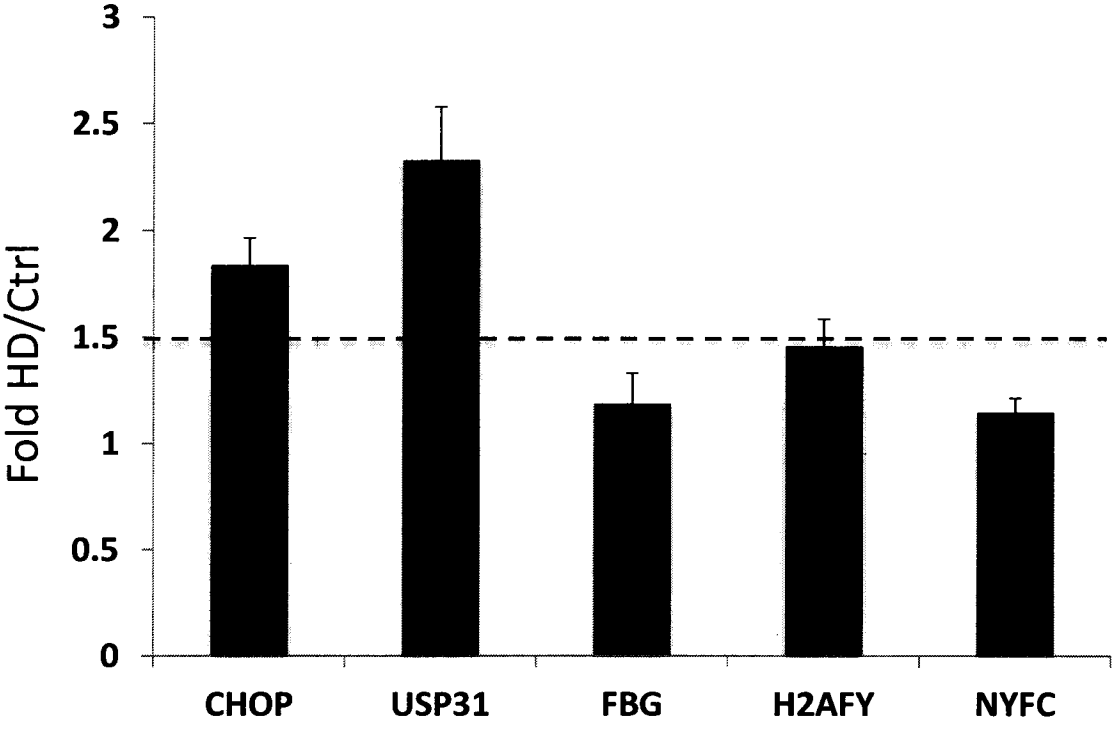
10
8
6
4
2
0
CHOP
USP31
FBG
H2AFY
NYFC

Fig. 4


Fold HD/Ctrl
3
2.5
2
1.5
1
0.5
0
CHOP
USP31
FBG
H2AFY
NYFC

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/021357

A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/11(2006.01)i, C12N 15/63(2006.01)i, C12N 15/79(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N 15/11; C40B 30/06; C40B 60/00; A61K 31/7105; C12Q 1/68; C12N 15/63; C12N 15/79

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: promoter, USP31, gamma-FBG, H2AFY, NYFC, CHOP, DDTI3, 38A locus, 17A

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012-0100541 A1 (ORDWAY, JARED et al.) 26 April 2012 See abstract; paragraphs [0123]-[0124]; claim 1; SEQ ID NO: 261.	1-3,6
A		4-5,7-10
X	US 2009-0018031 A1 (TRINKLEIN, NATHAN et al.) 15 January 2009 See abstract; paragraph [0134]; claims 1 and 10; SEQ ID NOs: 1503 and 2758.	1-3,7-8
A		4-6,9-10
X	US 2009-0023674 A1 (PAGANO, ALDO) 22 January 2009 See abstract; claims 22, 25, 31 and 32; SEQ ID NO: 72.	1-3,9
A		4-8,10
A	NCBI, GenBank accession no. AC002040.1 (30 October 2002) See the whole document.	1-10
A	NCBI, GenBank accession no. DM061108.1 (30 March 2009) See the whole document.	1-10
A	NCBI, GenBank accession no. HV321056.1 (15 July 2011) See the whole document.	1-10

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2014 (25.08.2014)	**26 August 2014 (26.08.2014)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701,
Republic of Korea
Facsimile No. +82-42-472-7140

Authorized officer
HEO, Joo Hyung
Telephone No. +82-42-481-8150

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/021357

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010-0304384 A1 (VALDIVIESO AMATE, FERNANDO et al.) 02 December 2010 See abstract; claim 1.	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/021357

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 28
because they relate to subject matter not required to be searched by this Authority, namely:
Claim 28 pertains to methods for treatment of the human body by therapy, and thus relates to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

2. ☒ Claims Nos.: 12-14,16,18-21,23-24
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 12-14, 16, 18-21 and 23-24 are unclear since they are referring to the multiple dependent claims which do not comply with PCT Rule 6.4(a).

3. ☒ Claims Nos.: 11,15,17,22,25-29
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

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